

On the conflict between precision and robustness in the proportion regulation of cell types

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Abstract

In *Dictyostelium discoideum* the proportion of cell types is known to be actively regulated, recovering, for example, after the removal of a given cell type. However, we have recently shown that regulation is intrinsically imprecise: it controls the proportion above/below certain upper and lower thresholds, but not within an allowed range of values that these thresholds define. To explain this finding we present a model based on (i) a global negative feedback, and (ii) a cell-autonomous positive feedback leading to a hysteresis-like behaviour (i.e, a bistability of cell type). The simulated cell-type proportion is indeed found to span a range of values as a consequence of the bistability. We conclude that there is a general conflict between the precision of proportion regulation and the robustness of the differentiation of cell types.

Tissues of multicellular organisms are formed by a variety of cell types in fixed proportions. Both the normal maintenance of these tissues and their restoration after injury involve transdifferentiation, dedifferentiation and/or differentiation of pluripotent cells (the so-called stem cells) into finally differentiated cell types [1]. Examples of major biomedical relevance include the blood cell system and the epidermis. While the molecular details of the underlying cell-cell signalling are now being unveiled [2], the global mechanisms that regulate the populations remain obscure. Some theoretical investigations on proportion regulation have been conducted [3, 4], but their scope has often been too abstract/complex for comparison with experimental data or otherwise too detailed to give conceptual insights. Here we present a simple model, based on recent experimental findings for the regulation of cell types in the slime mould *Dictyostelium discoideum*.

In *Dictyostelium* a multicellular aggregate, the mound, is formed by the cAMP-mediated aggregation of $10^2 - 10^5$ cells. This mound elongates into a migrating cylindrical "slug", which under appropriate conditions transforms into a mushroom-like structure, the fruiting body, with a stalk composed of vacuolated, dead cells topped by a mass of spores. Cell type pattern in the slug is highly organized along an anterior-posterior axis (see Fig. 1). The anterior is composed of prestalk (pst) cells (which ultimately differentiate into stalk cells), with prestalk A cells (pstA) occupying the $\sim 10\%$ foremost tip of the slug and prestalk O cells (pstO) $\sim 15\%$ immediately following. The remaining $70 - 90\%$, the posterior region, is mainly made of prespore (psp) cells (which become spores) (see review [5]).

In slugs the qualitative cell type patterning is invariant over three orders of magnitude of volume [6]. Furthermore slugs can regenerate a normal cell-type pattern after removal of one of the cell types [7]. These observations led Bonner to propose

that the proportion of cell types is regulated to have a constant value [6]. Using an improved cell type marker, a β -galactosidase with 1 hour half-life under the control of the prestalk-specific promoter *ecmA*O, we have characterized the regeneration process with previously unattained accuracy [8] (see Fig. 1).

When we isolated the prestalk zones from large slugs, which originally contained $10.5 \pm 3.3\%$ prestalk cells, the prestalk proportion at the end of regeneration was $28.0 \pm 2.1\%$, significantly higher than their initial value. The initial proportion is thus not completely recovered. Moreover, removing a part of the prespore zone fails to trigger any regeneration at all as long as the amputated slug contains less than $\sim 30\%$ prestalk cells; the regeneration process is initiated, however, as soon as this threshold is exceeded.

From these results it follows the regulation is intrinsically imprecise: it appears to control the proportion above and below the 10%/30% thresholds, but not within the allowed range of values that these thresholds define.

Several studies have presented evidence that prestalk cells require a chemical secreted by prespore cells to remain in their prestalk differentiated state [9]. Based upon these facts, it has been proposed that a small diffusible molecule acting as prespore inhibitor might be regulating the proportion of cell types [10, 11, 12].

In contrast to previous suggestions [13], the overall pattern in the slug does not appear to be regulated by concentration gradients, as in Turing-type reaction-diffusion mechanisms, but by cell sorting. Thus (i) slugs don't seem to display any size-independent characteristic length [8]; (ii) cell sorting occurs faster than cell transdifferentiation; (iii) proportion regulation without spatial pattern has been observed [9]. Sorting itself appears to be oriented by periodic cyclic AMP signals propagating as waves from the tip [14]. It therefore appears to be justifiable to

use models in which gradients are neglected and the concentration of the prespore inhibitor is assumed to be homogeneous along the slug.

The model we develop here is based on Kay's [15]. We assume a global negative feedback meant to regulate the proportion. This feedback is mediated by a diffusible prespore inhibitor u which is produced by prespore cells at a rate k_u and degraded by prestalk cells at a rate $h_u(u)$. n_{psp} and n_{pst} are prespore and prestalk cell density, respectively. Cell type transdifferentiation is represented by the decreasing function $f(u)$ for $pst \Rightarrow psp$ conversion and the increasing function $g(u)$ for $psp \Rightarrow pst$.

$$\frac{du}{dt} = k_u n_{psp} - h_u(u) n_{pst} \quad (1)$$

$$\frac{dn_{pst}}{dt} = g(u) n_{psp} - f(u) n_{pst} \quad (2)$$

The degradation rate of u is approximated as $h_u(u) = h_u u$. Cell densities can be written as $n_{psp} = \rho(1 - \eta)$ and $n_{pst} = \rho\eta$, where ρ stands for the total cell density and η for the prestalk proportion. Equations can be nondimensionalized with the following change of variables: $t^* = \tau_u t$, $u^* = \frac{h_u}{k_u} u$, $f^*(u^*) = \tau_\eta^{-1} f(u)$, $g^*(u^*) = \tau_\eta^{-1} g(u)$. $\tau = \tau_\eta / \tau_u$, where $\tau_u = h_u \rho$ is the characteristic time of u degradation and τ_η the characteristic time of cell conversion. In the following we use the new variables without the asterisk.

$$\frac{du}{dt} = (1 - \eta) - u\eta \quad (3)$$

$$\tau \frac{d\eta}{dt} = g(u)(1 - \eta) - f(u)\eta \quad (4)$$

The main novelty of this model is the hysteresis-like behaviour introduced by

the assumptions on g and f , representing a cell-autonomous positive feedback in the differentiation process. First we assume that differentiation is bistable, i.e. $psp \Rightarrow pst$ transdifferentiation begins only at $u > u_2$, but the reverse conversion requires a decrease of u below a much lower threshold u_1 . Second, as illustrated by Fig. 2B, we assume that this behaviour applies not only for the single cell, but for the whole population (this can be shown to be the case as far as the distribution of thresholds u_1 and u_2 don't overlap). Then, if u is taken as an external parameter, it follows from the equation 4 that the proportion will display a hysteresis-like behaviour such as shown in Fig. 2A. Note that if f and g had been allowed to overlap, the steady state would be achieved at the expense of a continuous to-and-fro cell interconversion (not observed experimentally) and η vs. u wouldn't display hysteresis [12].

After the nondimensionalization, the equations have only 3 parameters: τ , u_1 and u_2 . The particular dependence of f and g on u doesn't appear to have any noteworthy effect on the regulation dynamics. Simulations have been performed assuming $f(u) = u_1 - u$ for $u < u_1$, otherwise zero, and $g(u) = u - u_2$ for $u > u_2$, otherwise zero.

Fig. 3 shows the phase plane of the system. A whole segment of fixed points is found for $u_1 < u < u_2$ and $\eta = \frac{1}{1+u}$. In consequence the range of stable proportions extends from $\eta_{min} = \frac{1}{1+u_2}$ to $\eta_{max} = \frac{1}{1+u_1}$. The experimental values of prestalk proportion in the slug (10 – 30% [8]) are satisfied for $u_1 = 2.4$ and $u_2 = 9$.

Perturbations in the proportion within the (η_{min}, η_{max}) interval result in an adjustment of u , but don't require the proportion to be recovered, in accordance to recent findings [8]. However, if the proportions are altered beyond η_{min} or η_{max} , proportion regeneration occurs after a time interval of u degradation/production τ_u (horizontal lines in phase plane) and a time interval of exponential-like increase/decrease

of the proportion τ_η (vertical curves). The parameter $\tau = \tau_\eta/\tau_u$ controls the ratio between these characteristic times. Simulations with any $\tau > 30$ give an excellent fit to experimental data of regeneration (see Fig 1B), suggesting that $\tau_u \ll \tau_\eta$. Such a result is fully consistent with observations that degradation time τ_u of putative regulators might lie in the $10^{-1} - 10^{-3}$ hours range, while the cell type transdifferentiation time τ_η can be estimated in the 1-5 hours range.

One might expect that after an increase/decrease in the proportion, the regulation would bring the proportion exactly to the lower/upper thresholds of the available range (η_{min}, η_{max}). However regeneration dynamics tends to overshoot beyond the thresholds. This overshooting might eventually lead to damped oscillations in prestalk proportion during regeneration. Being that such oscillations are both unrealistic and undesirable, they may give us a broad hint of the parameter ranges that natural selection would avoid. This is: small τ (i.e. similar time scale in signalling and cell type conversion) and narrow ranges of (u_1, u_2) (i.e. short distance between forward and reverse thresholds of u for conversion). Interestingly, this means that (at least some of) the parameters that would produce a quick and precise regulation of the proportion, would have a destabilising effect on the regulation dynamics. Current estimates of the parameters lie far from this oscillating regime.

In spite of the success of the model in reproducing the dynamics of proportion regeneration, a number of questions deserve further inquiry:

(a) The actual molecular basis of the negative feedback is still yet controversial. DIF-1 (Differentiation Inducing Factor-1), a chlorinated alkyl hexaphenone, is the main candidate molecule for the postulated prespore inhibitor/prestalk inducer [15]. However it has been recently found that it is necessary for *pstO* induction but not for *pstA* [17].

(b) The observation that η decreases with slug size [8, 12] can be related to the decrease of O_2 average concentration with size, which in turn may decrease production rate k_u .

(c) We have assumed that the u concentration is homogeneous along the slug. However, the diffusion length λ_u of putative regulatory molecules such as DIF-1 or cAMP can be estimated in the 0.15 – 1.5 mm range. Since effective diffusion will be even smaller, gradients may play a role in medium and big (~ 1.0 mm long) slugs. Being that the prespore inhibitor gradient is reversed, the difference in the transdifferentiation thresholds u_1 and u_2 becomes essential to stabilize the pattern.

(d) The transdifferentiation rates f and g for the population dynamics have been assumed based on the observation of hysteresis in cell type conversion. They should eventually be derived from positive feedback loops in the signal transduction pathways at the single cell level [5].

(e) This study has been solely concerned on the regulation between already differentiated cell types. It should be stressed that it may not be immediately extensible to the initial *Dictyostelium* cell type differentiation where positional effects may be important [16].

A general lesson may be drawn from this study. In tissue maintenance, it is important both to control the cell type proportions and to keep each of these cell types in a well differentiated state. However, there seems to be a conflict of between these two requirements. On the one hand proportion regulation is better served by signalling that involves fast global negative feedback. On the other hand, a robust cell differentiation requires a strong cell-autonomous positive feedback to operate the “switch” between cell types. Without positive feedback cell differentiation would result in a continuous spectrum of cell phenotypes. Yet, as a result of the positive

feedback, cell differentiation will always display some hysteresis in respect to the control exerted by the global regulative mechanism. This hysteresis poses a limit to the precision of the proportion regulation. In other words, it looks as though the more robust is the cell differentiation the less precise is the proportion regulation and vice versa. This conflict between precision and bistability appears to be a general property of systems of globally coupled bistable elements [4].

In spite of its sheer simplicity, we believe that the model proposed stands on reasonable experimental foundations and delivers new conceptual insights. In particular, it provides an explanation for recent findings on the imprecision of *Dicystostelium* cell type proportioning. In general, it illustrates the possible conflict between proportion regulation and robustness of cell differentiation in multicellular tissues.

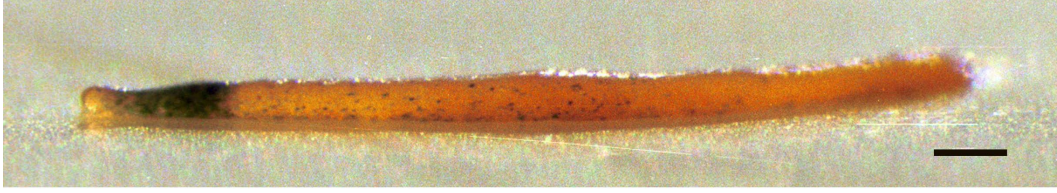
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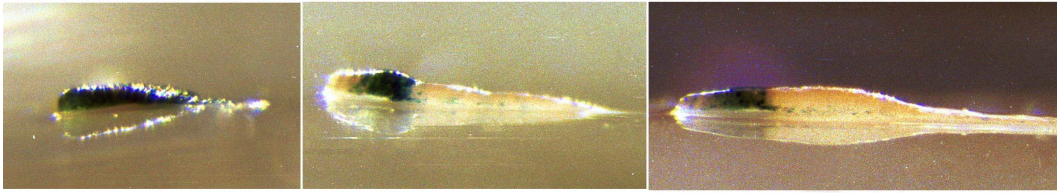
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Before cut



After cut



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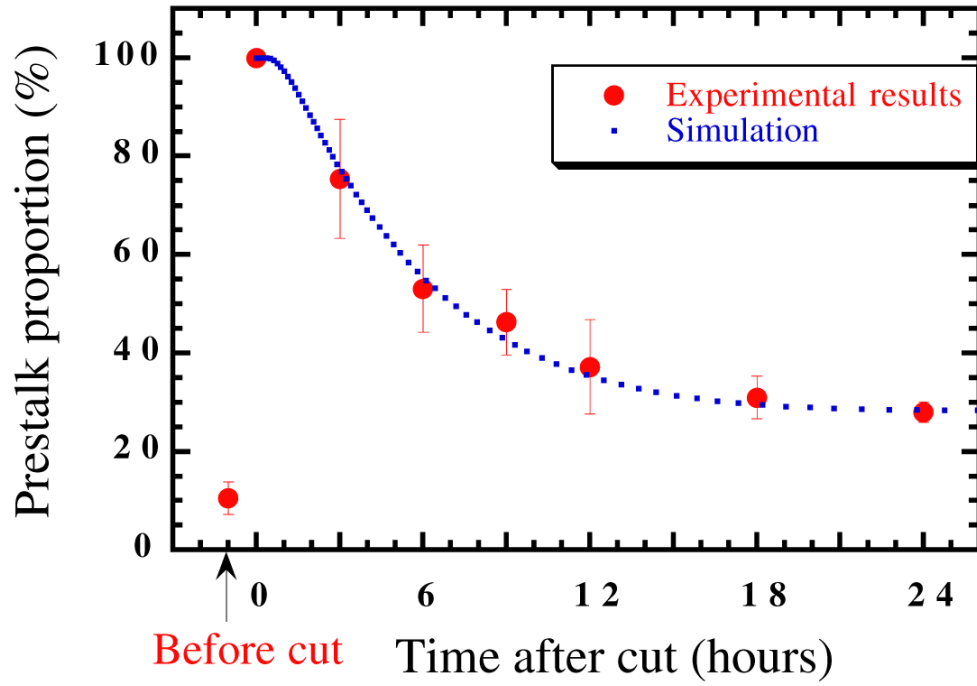


Figure 1: (Top panel) Slugs of *Dictyostelium discoideum* before and during regeneration after total removal of the prespore cells. (Bottom panel) Prestalk proportion before and during regeneration. Small squares show the results of simulation ($\tau = 30$, $u_1 = 2.5$, $u_2 = 9.0$, with $u(0) = 5.0$, $\eta(0) = 1.0$).

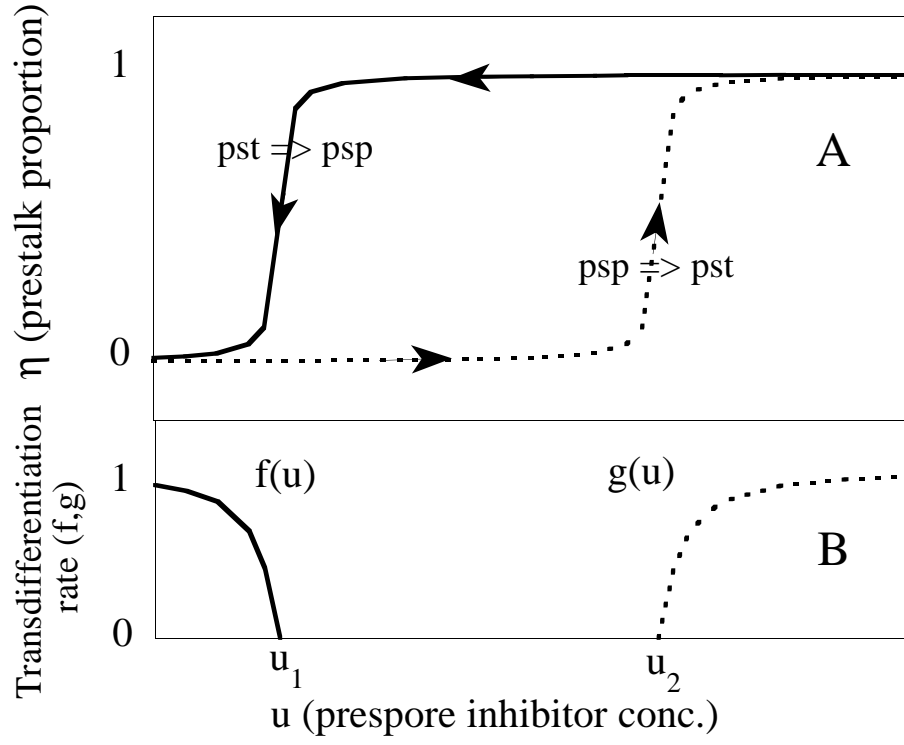


Figure 2: (A) Prestalk proportion dependence on the prespore inhibitor/prestalk inducer. The hysteresis-like behaviour can be seen as the fingerprint of bistability in cell type differentiation (B) Cell type transdifferentiation rates $f(u)$ and $g(u)$. It is postulated that f and g only take a positive value below (above) some threshold u_1 (u_2).

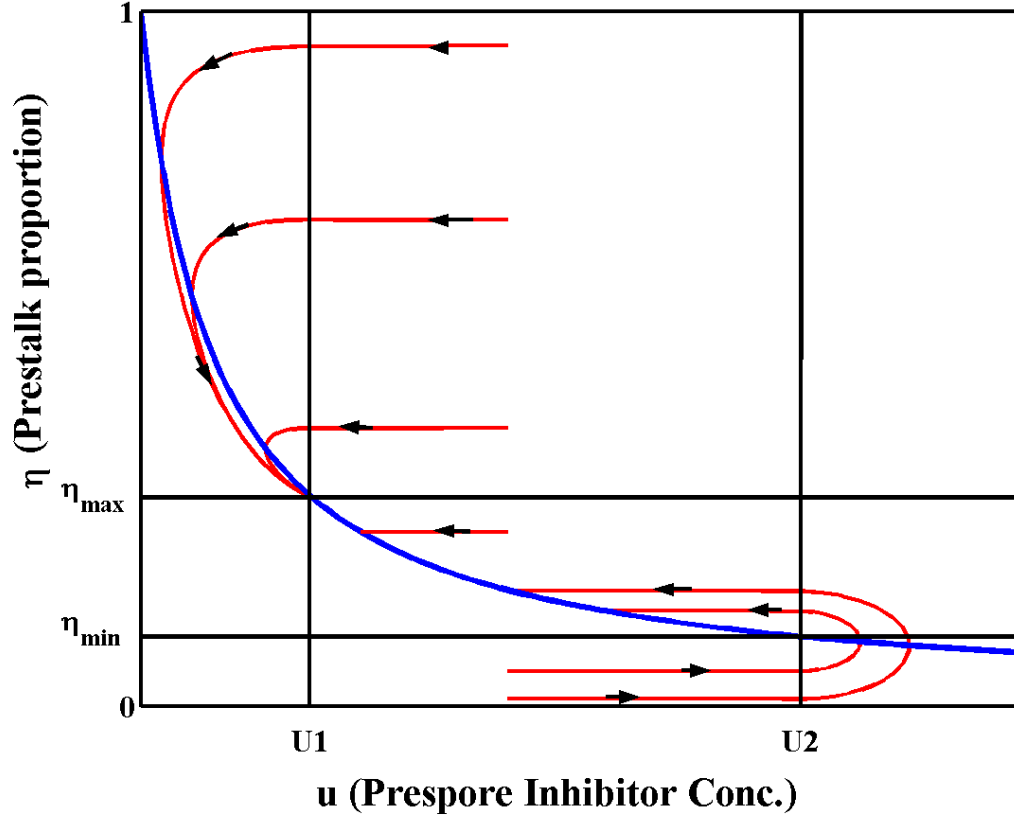


Figure 3: (A) Trajectories obtained from simulations starting at different initial conditions for $\tau = 30$, $u_1 = 2.3$, $u_2 = 9$ (red lines) . The nullcline $\frac{du}{dt} = 0$ is shown in blue. For initial proportions in the $\eta_{min} \leq \eta \leq \eta_{max}$, proportion doesn't change. However, when the system is perturbed beyond the upper/lower thresholds, the concentration of the regulatory molecule u decreases/increases until it induces transdifferentiation and the proportion returns to the allowed range.